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Effect of fertilising with pig slurry and chicken manure on GHG emissions from Mediterranean paddies

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Abstract

Soil fertilisation affects greenhouse gas emissions. The objective of this study was to compare the effect of different fertilisation strategies on N_2O, CH_4 emissions and on ecosystem respiration (CO_2 emissions), during different periods of rice cultivation (rice crop, postharvest period, and seedling) under Mediterranean climate. Emissions were quantified weekly by the photoacoustic technique at two sites. At Site 1 (2011 and 2012), background treatments were 2 doses of chicken manure (CM): 90 and 170 kg NH_4^+-N ha^{-1} (CM-90, CM-170), urea (U, 150 kg N ha^{-1}) and no-N (control). Fifty kg N ha^{-1} ammonium sulphate (AS) were topdress applied to all of them. At Site 2 (2012), background treatments were 2 doses of pig slurry (PS): 91 and 152 kg NH_4^+-N ha^{-1} (PS-91, PS-152) and ammonium sulphate (AS) at 120 kg NH_4^+-N ha^{-1} and no-N (control). Sixty kg NH_4^+-N ha^{-1} as AS were topdress applied to AS and PS-91. During seedling, global warming potential (GWP) was ~3.5-17% of that of the whole rice crop for the CM treatments. The postharvest period was a net sink for CH_4, and CO_2 emissions only increased for the CM-170 treatment (up to 2 Mg CO_2 ha^{-1}). The GWP of the entire rice crop reached 17 Mg CO_2-eq ha^{-1} for U, and was 14 for CM-170, and 37 for CM-90. The application of PS at agronomic doses (~170 kg N ha^{-1}) allowed high yields (~7.4 Mg ha^{-1}), the control of GWP (~6.5 Mg CO_2-eq ha^{-1}), and a 13% reduction in greenhouse gas intensity (GHGI) to 0.89 kg CO_2-eq kg^{-1} when compared to AS (1.02 kg CO_2-eq kg^{-1}).

Keywords: organic fertiliser, urea, ammonium sulphate, postharvest, seedling period; greenhouse gas emissions

1. Introduction

Globally, agriculture accounts for 60%, 50% and 1.1% of total anthropogenic N_2O, CH_4 and CO_2 emissions, respectively (Liu et al., 2012a). The GHG emissions from rice (*Oryza sativa* L.) and the subsequent global warming potential (GWP, 3757 kg CO_2-eq ha^{-1} $season^{-1}$) are roughly four fold those of wheat (*Triticum aestivum* L.; 662 kg CO_2-eq ha^{-1} $season^{-1}$) or maize (*Zea mays* L.; 1399 kg CO_2-eq ha^{-1} $season^{-1}$) (Linquist et al., 2012).

Flooded rice emits both N_2O and CH_4 due to the negative soil redox potential and different bacterial communities (Kögel-Knabner et al., 2010; Arends et al., 2014) as reported in both instantaneous and annual estimates (Brumme et al., 1999; Groffman et al., 2000). However, periods of N_2O consumption have been reported in many field studies (Brumme et al., 1999; Chapuis-Lardy et al., 2007; Van Groenigen et al., 2015; Wrage et al., 2004).

Anaerobiosis favours the activity of methanogens which in the presence of organic matter, substantially contribute to CH_4 emissions (Banik et al., 1996). Fertilisation management, mainly the type of N fertilisers, is a key factor in the control of N losses to the atmosphere and GHG emissions from paddy fields (Gogoi and Baruah, 2012; Maris et al., 2016). Urea is prone to large gaseous losses, particularly due to ammonia volatilisation (Mikkelsen et al., 1978) and denitrification to N_2O and N_2, though reports on N_2O emissions are contradictory (Lindau et al., 1991; Zou et al., 2005), possibly due to the influence of location and management practices (Zhao et al., 2011). Recent rice field studies suggest that high NH_4^+-N may stimulate CH_4 oxidation reducing its emissions (Bodelier and Laanbroek, 2004; Banger et al., 2012) by roughly 30 to 50% (Xie et al., 2010; Yao et al., 2012). It has been stated that when fertilising with ammonium sulphate the competition of sulphate-reducing bacteria for hydrogen reduces

CH_4 emissions. Nitrogen fertilisation may also increase CH_4 and CO_2 emissions due to increased rice biomass which can facilitate gas transport through rice plants (Dick, 1992; Iqbal et al., 2009; Singh et al., 1999; Wilson and Al-Kaisi, 2008), as well as enhance carbon substrate availability for methanogens (Lu et al., 2000; Schimel, 2000). Lindau et al. (1991) found that urea addition increased CH_4 emissions by approximately 40 to 75% compared to control. According to Hou et al. (2000), efforts to reduce the overall GWP of rice should focus on reducing CH_4 emissions. However, both CH_4 and N_2O need to be considered, as many strategies that reduce CH_4 emissions tend to increase N_2O emissions and vice-versa (Cai et al., 1998; Ma et al., 2007; Zou et al., 2005).

Soil CO_2 emissions are increasingly important as they integrate all the components of soil CO_2 production, including rhizosphere respiration and soil microbial respiration (Iqbal et al., 2009), one of the primary fluxes of C between soil and atmosphere. Almaraz et al. (2009) did not observe any significant effect of mineral fertiliser application on cumulative CO_2 emissions.

Nowadays, urea and ammonium sulphate account for about 90% of the total N fertiliser applied to rice cultivation in the world (Food and Agriculture Organization, 2013). The autonomous regions of Aragon and Catalonia hold about 42% of the Spanish pig herd (MAGRAMA, 2014), Catalan pig farms representing 29% (IDESCAT, 2016). Furthermore, in Catalonia, the Montsià (Ebro Delta) county concentrates 32% of the poultry farms of this region, with approximately 2 million heads producing 51,786 t manure year^{-1} (MAGRAMA, 2014). The use of pig slurry (PS) and chicken manure (CM) as fertiliser to winter cereal and maize is the most common recycling method. It is a challenge to apply PS and CM at an optimised dose also to rice crops, this not yet being common.

Field measurements of GHG emissions are typically limited to the rice crop. Some authors have pointed out the need to quantify annual rather than seasonal emissions (Fitzgerald et al., 2000; Liang et al., 2007) because of the importance of emissions during fallow periods. Furthermore, according to some Chinese studies (Ma et al., 2012) the rice seedling period (SP) had a GWP (of N_2O and CH_4) equivalent to the GWP of the rice crop, though there are few such studies (Ma et al., 2013). The cumulative N_2O emissions of the rice SP ranged from 0.24 to 0.62 kg N_2O-N ha^{-1} and that of CH_4 from 21.8 to 162.2 kg CH_4 ha^{-1} in Chinese systems (Liu et al., 2012a, Ma et al., 2013). In China, the rice seedlings are generally grown in nursery patches for 30-40 days before being transplanted into paddy fields, while in Mediterranean areas rice is mainly sown on site. Greenhouse gas emissions estimates should reflect the specific conditions of the different countries and the agricultural practices involved (IPCC, 2007).

In the European Union about 475,000 ha are devoted to rice (*Oryza sativa* L.) with a total production of 3.2 Mt of rice grain (1.8 Mt white rice). The European Union (EU) is a net steadily growing rice importer (some 809 400 t $year^{-1}$). Italy is the largest producer, with 52% of the total, followed by Spain with 28%. In Spain, the rice sector has a 259.03 million euros worth production (estimated for 2015). Spain is a net rice exporter (about 137 231 t $year^{-1}$ net exports). In Spain, rice paddies are localised in saline areas with environmental restrictions (Deltas and marshlands belonging or close to natural parks), and with waterlogged soils. This is also the case in some other Mediterranean areas such as Valencia (Spain) or the Camargue (France) regions. The main rice producing region in Spain is Andalucia (36.7% of the Spanish rice producing area; MAGRAMA, 2015), Catalonia together with Aragon hold 24.7% of the Spanish rice area, followed by Extremadura (22.4%) and Valencia (13.8%). The Ebro Valley extends over 85,362 km^2 in NE Spain, including Aragon and Catalonia. It is one of the most intensively irrigated river basins in Europe (0.8 million ha of irrigated land; Wriedt et al., 2008). Soils near the rivers are classified as Fluvisol Eutric (FAO, 1974), while in the rest of the irrigated areas the most common soil types are Xerosol Gypsic and Xerosol Calcic. These soils are often salt-affected (CHE, 2008). About 86% of the cultivated Ebro Delta is, at present, under rice cultivation due to the presence of saline groundwater over large areas (Casanova, 1998), and to environmental policies (EU Regulation 1765/92; NATURA 2000 network and the Ramsar Convention).

Sowing density ranges from 160 to 200 kg seed ha^{-1}. On site, mechanical sowing (rice is not transplanted) takes place from April 15th to May 15th. A water layer of 3 to 5 cm is kept on the field after sowing. Later on it is increased to 10 to 15 cm. Flooding is constant throughout the rice cycle (with water running in and out of the fields at all times), except for the time when some agricultural practices (fertiliser side-dressing, pesticide treatments) require drainage. In early September, water is drained and rice can be harvested up to mid-October.

The objective of this study was to compare the effect of organic N fertilisers (pig slurry (PS) and chicken manure (CM)) with urea and ammonium sulphate on the emissions of N_2O, CH_4 and CO_2 from the rice paddy during the rice crop and for the postharvest (fallow) period, as well as for the seedling period.

Two contrasted sites in the Ebro Valley were studied, with different organic fertilisers available (CM or PS) and with different mineral N applied at sowing time (urea or ammonium sulphate). Both fertilisation strategies (mineral and organic) were designed to include a similar background applied NH_4^+-N dose (from 90 to 170 kg NH_4^+-N ha^{-1}) but to differ widely in organic-C application (CM had a high C/N ratio and PS a low one).

2. Materials and methods

2.1. Site description and experimental design

The experiment was carried out at two rice paddies located at two different sites in the Mediterranean Ebro Valley (NE Spain). Soils of the two sites are representative of Mediterranean and Spanish soils under rice. Site 1 is representative of the alluvial soils from deltas and river terraces and similar landforms: they are from moderate coarse textures up to moderate fine textures (Casanova et al., 2002). Salinity is highly variable and they have an organic matter content around 2%. Site 2 represents paddy soils from inland. Usually they are salt affected soils (Herrero and Aragüés, 1988; Nogués et al., 2000) cropped with rice to be reclaimed; they are more silty and with lower organic matter content. In all cases, tillage, before sowing, is done at high soil water content in order to create an impermeable layer in order to avoid seepage or saline water capillary rise. Site 1 is located at the Institute for Food and Agricultural Research and Technology (IRTA), at its Amposta station (coordinates: 40°42′30″N, 00°37′56″W, altitude: 3 m a.s.l.) in the Ebro River Delta (Fig. 1). The location is characterised by a Mediterranean climate, with an average annual temperature of 17°C and an average annual rainfall (over the last 10 years) of 550 mm, summer being the driest and hottest season of the year (rainfall below 43 mm and temperatures rarely higher than 26°C). In 2011 the average annual temperature was 16.9°C and the average annual rainfall was 495 mm, while in 2012, the average annual temperature was 16.4°C and the an average annual rainfall was 519 mm. In both years, the summers were neither noticeably warmer nor wetter than the previous 10 year average.

Site 2 is located at Villanueva de Sigena (coordinates: 41°45′32″N, 0°2′18″W, altitude: 297 m a.s.l.) (Fig. 1). The location is characterised by a semi-arid climate, with an average annual temperature of 14.6°C and an average annual rainfall (over the last 10 years) of 350 mm, summer being the driest and hottest season of the year (rainfall below 20 mm and temperatures higher than 30°C). In 2012 the average annual temperature was 14.3°C and the average annual rainfall was 375 mm. In 2012, the summer was neither noticeably warmer nor wetter than the previous 10 year average. The main soil properties and climatic characteristics of these sites are summarised in Table 1. Site 1 was sampled in 2011 and 2012. Site 2 was sampled in 2012 (Table 2). Rainfall and air temperature during the sampling period (Figs. 2a and b) were obtained from the closest meteorological stations to the experimental sites (Meteorological Service of Catalonia: http://www.ruralcat.net/web/guest/agrometeo.estacions, Site 1; and Meteorological Service of Aragon: http://eportal.magrama.gob.es/websiar/Inicio.aspx, Site 2). Additionally, soil temperature (10 cm depth) was measured when soil samples were taken.

At both sites, a water layer of 3 to 5 cm was kept on the field during the seedling period (the first 35 days after sowing) and of 10 cm from the end of the seedling period to harvest. Water was continuously flowing in and out of the plots at both sites.

The dates of the main field labours and of the sampling periods are detailed in Table 2. At Site 1, rice (*Oryza sativa* L.), cultivar Gleva, was sown directly on site in 2011 and 2012 (indicated on the treatment name as -11 and -12) at a density of 182 kg ha^{-1}. The background treatments were (Table 3): urea (U-11 and -12) 150 kg N ha^{-1}, 2 different doses of CM containing 90 (CM-90-11 and -12), and 170 kg NH_4^+-N ha^{-1} (CM-170-11 and -12); and a control with 0 kg N ha^{-1} (Control-12) as well a background 0 kg N ha^{-1} with ammonium sulphate topdress application (AS-top-11 and -12) (Table 3). In both years, ammonium sulphate (AS) was applied as topdress fertiliser at a dose of 50 kg N ha^{-1} to all the treatments except for the control (Table 3). The control treatment was not sampled in 2011. Thus, there were four treatments in 2011 and five treatments in 2012.

The treatments were randomly distributed in three blocks (replicates) (Fig. 3a). One static chamber per block of the studied treatments was sampled (12 chambers in 2011, and 15 chambers in 2012). The dose of organic-N applied each year with the CM is shown in Table 3. The main properties of the CM applied are shown in Table 4. The chicken manure applied in 2011 contained 17.8 kg N t^{-1}, which is comparable with the 15.0 kg N t^{-1} content of chicken manure stated in the regulation (Decree 136/2009 of the Catalan Government). The chicken manure applied in 2012, however, was much dryer and though it was poorer in ammonia the applied material contained 33.7 kg N t^{-1}. The area of each individual plot was 20.25 m^2 (4.5 m $*$ 4.5 m). Sampling began in May 2011 and finished in January 2013, including both the rice crop and the postharvest period of 2012 and partly that of 2013 (Table 2).

At Site 2, rice (*Oryza sativa* L.), cultivar Guadiamar, was sown directly on site in 2012 at a density of 150 kg ha^{-1}. The background treatments were (Table 3) ammonium sulphate (AS-12) at a dose of 120 kg NH_4^+-N ha^{-1}, 2 doses of PS equivalent to 91 (PS-91-12), and 152 kg NH_4^+-N ha^{-1} (PS-152-12) plus a control (no nitrogen applied). Topdressing at a dose of 60 kg NH_4^+-N ha^{-1} was applied only to the AS-12 and PS-91-12 treatments.

Treatments were established to obtain a potential range of GHG losses, according to available field practices during the seedling stage. Minimum losses were expected from Site 2, due to the lower clay content and fertilisers used: ammonium sulphate (it could reduce CH_4 emission through the competition by sulphate-reducing bacteria for hydrogen) and pig slurry because of its lower C/N ratio (Table 3). A range of applied NH_4^+-N from 90 kg N-NH_4^+ ha^{-1} up to 170 kg N-NH_4^+ ha^{-1} (about twice the minimum) to quantify the effect of ammonium from different organic sources on GHG emissions was established.

The main properties of the PS applied are shown in Table 4. The applied pig slurry contained 4.0 kg N m^{-3}, which is comparable with the 4.4 kg N m^{-3} content of chicken manure stated in the regulation. Thus, there were four treatments. The fertilisation treatments were randomly distributed in four blocks (replicates) (Fig. 3b). One static chamber per block of the studied treatments was sampled (16 chambers). The area of each individual plot was 36 m^2 (6 m * 6 m) for PS-91-12 and PS-152-12 and 18 m^2 (3 m * 6 m) for AS-12 and the control (C-12) treatments.

The numbers of static chambers used are in line with those of Ma et al. (2012), Liu et al. (2012a), Ali et al. (2015) Adviento- Borbe and Linquist (2016) who sampled three or four replicates per treatment whit one chamber per replicate.

Sowing was earlier on the coast (Site 1) with respect to inland (Site 2) (Table 2) as this favours yield (Casanova et al., 2000). In both sites, weeds and pests were controlled in accordance with local conventional practice.

2.2. Gas sampling and quantification

Gas samples were collected weekly using the static closed chamber method (Ma et al., 2012; Liu et al., 2012a; Sander and Wassmann, 2014) throughout the rice crop, at both sites. The static chamber method is the conventional approach for determining greenhouse gases (GHG) emissions from rice fields (Sander and Wassmann, 2014). However, there is no commonly accepted standard for the measurement protocols, and therefore published studies encompass a variety of different modalities depending on the specific settings and labour limitations of a given study (Sander and Wassmann, 2014). In the present study, the cylindrical (20 cm diameter and 60 cm high) static chambers were made of polyvinyl chloride (PVC) coated with an epoxy resin. They were inserted 18 cm into the soil. This cylinder was closed with a vented screwed lid with a three-way key. Air samples from inside the chambers were taken in triplicate immediately after closing the chamber, and 20 and 40 min later. Samples were taken through a Teflon® tube connected to the three-way key and into 100 ml plastic syringes, adapted with a valve. Air inside the chamber was mixed by filling and emptying the syringe six times before withdrawing the sample. After taking the air sample, the syringes were closed with the valve. After the last sampling (40 min from closing the chamber), the three-way keys were left open.

The syringes were transported to the laboratory and the concentrations of N_2O, CH_4 and CO_2 in the air sampled were quantified using the photoacoustic technique (Innova 1412 Photoacoustic Multigas Monitor). In this study, the standard error of the average cumulative greenhouse gases emissions was selected as the statistic of the error. The standard error of the gas emissions includes both field sampling error and laboratory analysis error.

Surface soil temperature was always recorded during sampling. The photoacoustic analyser refers the gases concentration to 20ºC and 1 atm; for the gas concentration was corrected to refer to the actual field temperature and atmospheric pressure of each sampling day. Sampling undertaken done at the time of the day when soil temperature was about the average soil temperature of the day in order to minimise over- or underestimation of the emissions caused by daily soil temperature variation.

Since the chambers were not transparent, it can be assumed that the CO_2 flux was the ecosystem respiration including plant autotrophic respiration. Since the plants were inside the closed chambers it was assumed that the stomata were closed in the darkness.

2.3. Calculations and statistical analysis

The N_2O and CH_4 emissions fluxes and ecosystem respiration (CO_2) were determined from the linear increase of gas concentration at each sampling time (0, 20 and 40 min) during the time of chamber closure. The cumulative emissions throughout the study period were calculated by integrating the emissions curve over time.

Emissions were calculated for different periods: the rice crop, and additionally for the postharvest and seedling periods. The rice crop lasts from the end of the seedling period (day 35 after sowing) until harvest. The postharvest (fallow) period extends between harvest (September) and the following sowing (April). In 2011, the postharvest (fallow) period was sampled entirely, while in 2012 it was sampled until January 2013 when the research project ended (Site 1). The emissions during the seedling period (SP) (35 days after sowing) were calculated with the data from 2012 for Site 1. The seedling stage starts immediately after sowing and lasts until just before the first tiller appears. During this stage, the seminal roots and up to five leaves are developed. It lasts for a period of 35-40 days.

The direct N_2O emission factor (EF) was calculated according to IPCC (2001) as the difference between the N_2O emitted from fertilised soil and that from the control divided by the N applied as fertiliser throughout the rice crop. The normal distribution of the flux data (N_2O, CH_4 and CO_2) was verified using the Shapiro-Wilk test; this was carried out with the JMP 9 statistical software (SAS Institute, 2010). When necessary, in order to fulfil the assumption of normality, data were log transformed prior to analysis. The cumulative emissions of N_2O, CH_4 and ecosystem respiration (CO_2) were examined using data from all plots and an analysis of variance was performed with the Statistical Analysis System (SAS) software (SAS Institute, 2014). Significant differences among treatment means were further examined by Tukey's multiple range tests at the 0.05 probability level. Data in the tables and figures are presented as average values ± standard errors.

2.4. Global warming potential (GWP) and greenhouse gas intensity (GHGI)

Global warming potential (GWP) is an index defined as the cumulative radiative forcing between the present and some chosen later time ‘‘horizon’’ caused by a unit mass of gas emitted now. In GWP estimation, CO_2 is typically taken as the reference gas, and an increase or reduction in emissions of CH_4 and N_2O is converted into ‘‘CO_2-equivalents’’ through their GWPs. Therefore, in the present study, the GWPs of N_2O and CH_4 emissions were calculated in units of CO_2 equivalents (CO_2-eq.) over a 100-year horizon (DeForest et al., 2007). A radiative forcing potential relative to CO_2 of 298 was used for N_2O and of 25 for CH_4 (DeForest et al., 2007). Although soil CO_2 fluxes also represent a source of GHG emissions, on a global scale they are largely offset by high rates of net primary productivity and atmospheric CO_2 fixation by crop plants, and are therefore estimated to contribute <1% to the GWP of agriculture (Smith et al., 2008; Linquist et al., 2012). Therefore, CO_2 as a contributor to GWP was not included in this analysis. The GWPs of N_2O and CH_4 emissions were calculated using the following equation (IPCC, 2007): GWP (kg CO_2-eq ha^{-1}) = cumulative N_2O emissions * 298 + cumulative CH_4 emissions * 25.

At physiological maturity, rice ears were harvested (1 to 4 m^2) and grain humidity was determined after drying it to a constant weight at 65°C in order to calculate the rice yield adjusted to 14% grain moisture content.

The greenhouse gas intensity (GHGI; kg CO_2-eq kg^{-1} grain yield) was calculated as follows: GWP (kg CO_2-eq ha^{-1}) / grain yield (kg grain yield ha^{-1}) (Qui et al., 2010; Shang et al., 2011; Liu et al., 2012a).

3. Results

3.1. Nitrous oxide emissions of the rice crop

At Site 1, as shown in Figs. 4a and b, all treatments had a similar pattern of N_2O emissions during the rice crop. The N_2O fluxes ranged from -370 (in 2011) to 318 g N_2O-N ha^{-1} d^{-1} (in 2012) and some high peaks occurred after AS was topdress applied (day 42 of sampling in 2011 and day 70 of sampling in 2012). The average of the N_2O fluxes measured was: -15, -9, 2 and 5 g N_2O-N ha^{-1} d^{-1} for AS-top-11, U-11, CM-170-11 and CM-90-11, respectively. In 2012, they were 2 g N_2O-N ha^{-1} d^{-1} from control and ranged from 7 to 11 g N_2O-N ha^{-1} d^{-1} for the other treatments.

At site 1, the N_2O emission factor (EF) ranged from 0.86 to 7.3% of the N applied (Table 6), with the maximum value for AS-top-12, and was about 2% for the CM-90-12 and U-12 treatments.

At Site 2, high fluxes of N_2O were measured after background fertilisation with the high dose of PS and with AS, but they were lower (than the control) with the low PS dose (Fig. 4c). Nevertheless, the average N_2O fluxes during the rice crop were close to 2 g N_2O-N ha^{-1} d^{-1} for all treatments, and the cumulative N_2O emissions did not differ among treatments (Table 5). The EF was negligible for AS-12 and PS treatments.

Overall, the cumulative N_2O-N emissions during the rice crop ranged from 0.96% (AS-12) to 1.35% (PS-91-12) of the N applied.

3.2. Methane emissions of the rice crop

At Site 1, the average daily CH_4 fluxes in the two years varied from -7.19 to 24.62 kg CH_4 ha^{-1} d^{-1} (Fig. 5). In 2011, the fluxes were lower than in 2012. The cumulative CH_4 emissions were increased by U and CM fertilisers (Table 5).

At Site 2, the average daily CH_4 fluxes were between 0.2 and 0.3 kg CH_4 ha^{-1} d^{-1} and significantly ($p<0.05$) differences in cumulative emissions between the treatments and the control were found (Table 5).

3.3. Carbon dioxide flux (ecosystem respiration) of the rice crop

At Site 1, in 2011, the fluxes of CO_2 reached a maximum during the reproductive stage of rice (days 45 to 80 of sampling), and then declined until the final drainage (Fig. 6a). The average daily CO_2 fluxes varied from 12 to 19 kg CO_2 ha^{-1} d^{-1} (Fig. 6). In 2012, the average of the daily fluxes of CO_2 increased from 6 up to 16 kg CO_2 ha^{-1} d^{-1} as N dose increased. Differences in the cumulative CO_2 respiration were only recorded in 2012 (Table 5), being larger in CM-170-12 than in the U-12 and C-12 treatments at Site 1. At Site 2, in 2012, the average daily CO_2 respiration fluxes ranged from 7 to 12 kg CO_2 ha^{-1} d^{-1}; and significant ($p<0.05$) differences between treatments in the cumulative CO_2 ecosystem respiration were found (Table 5).

3.4. Global warming potential (GWP) and greenhouse gas intensity (GHGI) of the rice crop

The contribution of CH_4 to total GWP was always much larger than that of N_2O throughout the rice crop. At Site 1, the maximum GWP was calculated for U-12 (17 Mg CO_2-eq ha^{-1}) and CM where it ranged from 14 (CM-170-12) to 37 (CM-90-12) Mg CO_2-eq ha^{-1}. At Site 1, the GWP was negative for the AS-top-11, U-11 and C-12 treatments (Table 6). At Site 2, the maximum GWP reached only 6 to 8 Mg CO_2-eq ha^{-1} from the AS-12, PS-91-12 and PS-152-12 treatments (Table 6).

The highest GHGI (~ 5 kg CO_2-eq kg^{-1}) was calculated for Site 1 and was associated, in 2012, with CM treatments (Table 6).

3.5. Greenhouse gas emissions during the postharvest period

In both years, at Site 1, during the postharvest (fallow) period no notable N_2O emissions were observed (Figs. 4a and b). In both years, the CH_4 flux dropped rapidly, within a few days from the final drainage before harvest (Figs. 5a and b). The cumulative CH_4 fluxes were negative, from -416 to -23 kg CH_4 ha^{-1} (Figs. 5a and b), without differences between treatments (Table 7). Soil Eh during the postharvest period ranged from -109 to +112 mV (Fig. 5d). The ecosystem respiration (CO_2 flux) ranged from -13 (in 2011) to 2001 kg CO_2 ha^{-1} from the CM-170-12 treatment (Table 7). Emissions were lower than those of the rice crop.

3.6. Greenhouse gas emissions during the seedling period

At Site 1, the cumulative N_2O emissions during the rice seedling period were significantly ($p<0.05$) affected by the fertilisation treatment (Table 8). They were significantly ($p<0.05$) larger (roughly four times) with CM (~3 kg N_2O-N ha^{-1}) than from U, while U did not differ with the control. The cumulative N_2O emissions during the SP represent between 8 to 37% of the cumulative N_2O emissions during the rice crop (Table 8).

There were clear peaks of CH_4 (days 14 and 19 of sampling) from the CM-90-12 treatment during seedling (Fig. 5b). Application of CM significantly ($p<0.05$) increased CH_4 emissions, although they decreased as the dose of CM increased (Table 8). They ranged from -19 kg CH_4 ha^{-1} for U-12 to 418 kg CH_4 ha^{-1} for CM-90-12. For manures, they accounted for between 38% (CM-90-12) and 55% of the total CH_4 emissions during the rice crop (Table 8).

The GWP did not differ between the control and U (where GWP was negligible) treatments, but was significantly ($p<0.05$) higher for CM ranging from 5.5 (CM-170-12) to 11.5 Mg CO_2-eq ha^{-1} (CM-90-12). The cumulative ecosystem respiration (CO_2 flux) tended to increase with the CM dose, being higher for the CM-170-12 treatment (2.4 Mg CO_2-eq ha^{-1}) than for the U-12 treatments and the control (~460 kg CO_2 ha^{-1}). The cumulative CO_2 emissions accounted for 12% and up to 16% of those recorded for the rice crop for the CM-90-12 and CM-170-12 treatments and for just 5% and 6% for the control and U-12 rice crop emissions, respectively (Table 8).

At Site 2, fertilisation treatments only affected the cumulative N_2O emissions (Table 8). The PS-152-12 treatment (0.9 kg N_2O-N ha^{-1}) was the only one with emissions higher than the control (0.4 kg N_2O-N ha^{-1}). The cumulative N_2O emissions during the SP represent between 15 to 41% of the cumulative N_2O emissions during the rice crop (Table 8). The GWP varied from 986 (PS-91-12) to 1996 kg CO_2-eq ha^{-1} (AS-12; Table 8). The ecosystem respiration (~114-197 kg CO_2 ha^{-1}) was less than 3% of that of the rice crop (Table 8).

4. Discussion

4.1. Nitrous oxide emissions during the rice crop

The two sites are not comparable and represent the two main types of conditions in which rice is cultivated in Spain. Both nitrification and denitrification are known to occur in flooded rice soil (DeDatta, 1995; Kyaw and Toyota, 2007) since they have an aerobic surface layer and an anaerobic subsurface one. In the present study, some oxygen exchange took place at both sites as water was running into and out of the plots continuously. Therefore N_2O emissions were probably also due to processes other than denitrification. There was an increase of N_2O emissions from the organic fertiliser treatments (CM at Site 1) with respect to the control, (Table 5), but higher the larger the organic-C applied with the organic fertiliser (Table 3). The results of the present study are in agreement with those of Das and Adhya (2014) and Baruah and Baruah (2015), who reported a similar trend when chicken manure was applied to paddy fields. For the other treatments, the low soil organic matter content (2.2% at Site 1 and 1.0% at Site 2) could have been a limiting factor for denitrification in the control and mineral treatments, and with the low PS dose at Site 2 due to its low organic matter content (Table 3). This constraint was also pointed out by Teira-Esmatges et al. (1998) and Menéndez et al. (2008). Vallejo et al. (2005) reported that Mediterranean agricultural soils often have organic contents below 2%, which is a limiting factor for denitrifying activity.

Maillard and Angers (2014), who made a meta-analysis of 42 research articles totalling 49 sites and 130 observations in the world, report that the number of studies using pig and poultry manures as fertiliser was very low and showed a very high variability, which limits the interpretation of the results. They suggested that more studies on the impact of pig and poultry manure were required, and that these should include C input data.

The soil acted as a net sink of N_2O during the first days of sampling and during the postharvest period, in both years. Negative fluxes of N_2O have previously been documented under various edaphoclimatic conditions for different crops (Chapuis-Lardy et al., 2007; Cardenas et al., 2010; Abalos et al. 2012). Past experiments have linked negative fluxes to soil properties (Heincke and Kaupenjohann, 1999; Khalil et al., 2002). However, the origin of net negative N_2O fluxes is often unclear (e.g. Chapuis-Lardy et al., 2007; Donoso et al., 1993; Goldberg and Gebauer, 2009). Possible pathways for N_2O consumption (negative fluxes of N_2O) have been reviewed in Van Groenigen et al. (2015).

In the present study, the background emissions of N_2O were (Table 5): 1.9 kg N_2O-N ha^{-1} both at Site 1 and at Site 2, higher than the background emissions from paddy fields in the literature (0.2-1.38 kg N_2O-N ha^{-1}, average: 0.79 kg N_2O-N ha^{-1}; Zou et al., 2007, 2009**)** and are most likely a consequence of mineralisation of previously incorporated rice straw (Gu et al., 2007).

The cumulative N_2O emissions from the fertilised treatments fall within the range reported by Akiyama et al. (2005), 1.0 to 6.2 kg N_2O-N ha^{-1}, based on 113 measurements from 17 continuously flooded sites worldwide. Also, Yao et al. (2010)

and Liu et al. (2012b) found that the standard error for continuously flooded fields with chemical or organic fertilizer application during the rice crop ranged from ±0.17 to ±1.03.

The N_2O emission factor (EF) of the N applied was negligible for AS and PS at Site 2, about 2 for U and CM-90-12 at Site 1, and up to 7.3 for AS topdress applied at Site 1, than the IPCC (2007) reference (i.e. 1% regardless of the N source, location, climate and soil type).

In general, the readily available topdress applied NH_4^+-N increased N_2O emissions (Fig. 4). This is in accordance with Linquist et al. (2003), Pathak et al. (2002) and Zou et al. (2005) who found that the N_2O emissions were between 3.43% and 60% higher than those of the control when topdress fertilising with mineral N.

4.2. Methane emissions during the rice crop

The cumulative CH_4 emissions in the present study (ranging from 98 to 972 kg CH_4 ha^{-1} ; Table 5) are in good agreement with the review of Minami (1995) and Yan et al. (2009), who found that the seasonal CH_4 emissions ranged from 48 to 1830 kg CH_4 ha^{-1} for paddy fields around the world. Also, the standard errors ranging from 8.92 to 104.18 (Table 5) are in agreement with the review of Shang et al. (2011) and Yao et al. (2013) (ranging from ±32.1 to ±121.23).

At both sites, U and AS tended to increase CH_4 emissions (decrease oxidation) and seemed to be affected by the dose of fertiliser (Table 5). These results are consistent with those of Lindau et al. (1991). In contrast, Cai et al. (1997) found that CH_4 emissions decreased with urea application, while Wang et al. (1993) reported no change in emissions with urea application.

Furthermore, at Site 1 the high dose of CM did not result in a further increase in CH_4 emissions and there was even a tendency to reduce CH_4 emissions. The reason for this is unclear; it may be due to the formation of phytotoxic substrates in the soil at high organic-C contents (Schütz et al., 1989; Kludze and DeLaune, 1995) which also inhibit plant development (Schütz et al., 1989). The same effect was observed by Khalil et al. (1998), who attributed it to a saturation effect for the production and release of CH_4, and therefore increments in fertiliser doses did not further increase CH_4 emissions. In the present study, this phenomenon was more evident in 2012 when more organic-N and C were applied, although the CH_4-C emitted as a percentage of the organic-C applied was almost the same in both years. This finding suggests that at the CM doses tested, the organic-C applied did not limit CH_4 emissions. En masse, the cumulative CH_4 emissions were inversely proportional to the CM dose and in both years significantly ($p<0.05$) higher than those of the control treatment and from the U treatment only in 2011 (Table 5). The higher soil NH_4^+ content for the CM-170 than for the CM-90 treatment (both years) resulted in the stimulation of methanotrophic activity and CH_4 oxidation, as Bodelier and Laanbroek (2004) and Noll et al. (2007) also described.

4.3. Carbon dioxide flux (ecosystem respiration) during the rice crop

In this study, the cumulative ecosystem respiration tended to increase with increasing doses of organic-C (Table 5). The CO_2-C emitted as a percentage of the organic-C applied was lower in 2012 than in 2011, indicating that the high organic-C dose in 2012 probably caused a “saturation” effect on methanogens. En masse, the respiration recorded was proportional to the CM dose, but only in 2012 a significant ($p<0.05$) difference between treatments was found (Table 5).

In both years, after the final drainage, a large peak of CO_2 was observed in all the treatments (Figs. 6a and b). One possible reason for this may be that the 10 cm water layer prevented oxygen diffusion into the saturated soil, resulting in limited aerobic respiration in the soil (Chimner and Cooper, 2003).

Although in the literature there is a high variability in GHG emissions and their errors using static chambers, the results of the present study are in line with those of Akiyama et al. (2005), Yan et al. (2009) and Liu et al. (2012a), Linquist et al. (2012), Sander and Wassmann (2014).

4.4. Greenhouse gas emissions during the postharvest period

In 2011, after the winter rains (Fig. 2a) when the remaining straw was slightly decomposed, water was drained from the field (Figs 4a, 5a, and 6a) and the straw was incorporated into the soil during February or March. From mid-March to the first half of April, land was prepared for sowing (levelled and fertilised and non-flooded). Unlike this pattern, in 2012 the field was drained for harvest on the 12th September and flooded again on the 28th September until the 29th November when it was drained until the end of sampling (3rd January 2013) (Fig. 4b), meaning that the soil was flooded during two of the three months of the postharvest period due to irrigation.

In both years, cumulative N_2O emissions were very low or negative and did not differ among treatments (Table 7), which could be associated with the reduction of N_2O to N_2 (Lardy et al., 2007).

In both years, the mineral N remaining in the soil after harvest (September) was very low in all treatments, though there was a nitrate peak in December (Figs. 7a and b). In both years, CH_4 fluxes decreased and remained low or even negative after harvest, the cumulative emissions being negative (Table 7), in agreement with Wang et al. (1998). The absence of a sufficiently reducing environment (Table 7), of plant-mediated transport, and lower temperatures than those of the rice crop can explain this fact.

During the postharvest period, after the final drainage (day 131 of sampling) soil Eh increased from -207 to 122 mV (Fig. 5d), which inhibited methanogenic bacteria (Jiao et al., 2006). From day 131 of sampling onwards, the soil acted as a net sink of CH_4 (Fig. 5b).

It is considered that CO_2 emissions come mostly from organic material decomposition and root respiration (Bowden et al., 1993). In both years, the cumulative ecosystem respiration during the postharvest period ranged from -13 (CM-90-11) to 2000 kg CO_2 ha^{-1} (CM-170-12), which was 99.9% and 87% lower than the same treatments during the rice crop (Table 7). Therefore, the postharvest period was not an important source of CO_2 with the exception of CM-170-12 to which high doses of C had been applied.

4.5. Greenhouse gas emissions during the seedling period

As already mentioned, the emissions during the seedling period (SP; the first 30 days following the sowing date) were calculated with data from 2012 for Site 1 and Site 2. Sowing was done directly “on site”, meaning that there was no seedling nursery and therefore no transplanting took place, as it is normal practice in Spain. The paddy soils were a small source of N_2O during the seedling period (SP; <0.9 kg N_2O-N ha^{-1}) when applying mineral fertiliser (U at Site 1 and AS at Site 2) or PS at Site 2, in line with the emissions and standard error obtained from Chinese rice paddies (e.g. Ma et al., 2012; Liu et al., 2012a), but they were much higher when CM was applied (3-4 kg N_2O-N ha^{-1}; Table 8), at Site 1 due to the different conditions and to the large amount of organic-C applied and to the weak initial anaerobic conditions (Fig. 5d). Ma et al. (2012) and Liu et al. (2012a) found that the N_2O emissions during the seedling period ranged from 0.10 to 0.98 kg N_2O ha^{-1} with a standard error ranging from ±0.01 to ±0.35 for paddy fields.

In accordance with other authors (Chadwick et al., 2000; Rochette et al., 2004; Vallejo et al., 2006), emissions increased in the days following organic fertilisation, and tended to increase with the dose of NH_4^+ from the PS and AS treatments at Site 2. As Vallejo et al. (2006) explained, although the amount of N_2O coming from nitrification is generally small, it can be large when NH_4^+-N is applied, as in pig slurry where it is the main form of N. The cumulative N_2O emissions from CM at Site 1 and from the PS-152-12 treatment at Site 2 were significantly ($p<0.05$) larger relative to control and U (Table 8). It has been reported that N_2O emissions are closely associated with the C/N ratio of the incorporated organic materials (Ma et al., 2007), and that a high C/N ratio (e.g. chicken manure) often decreases N_2O emissions, while N_2O emissions are generally facilitated by organic material with low C/N ratio (e.g. pig slurry) (Zou et al., 2005; Ma et al., 2007).

The cumulative CH_4 emissions during the SP were about half the cumulative CH_4 emissions during the rice crop from the CM-170-12 (Site 1) treatment to about 20% of them from the rest of the treatments at both sites. This differs from other studies, such as the one in China where Ma et al. (2012) found that CH_4 emissions during the SP were the same as during the rice crop, probably because of the intensive nursery patch system used there. Methane emissions were only increased in CM (Site 1) treatments compared to the urea treatment. Obviously, organic fertiliser provided methanogenic substrates and could promote CH_4 production during the rice SP period (Liu et al., 2012a). The cumulative CH_4 emissions during the seedling period in the present study (except for the CM-90-12 treatment, Table 8) are in good agreement with the review of Ma et al. (2012) and Liu et al. (2012a), who found that the cumulative CH_4 emissions during the same period ranged from 21 to 162 kg CH_4 ha^{-1} and, with the standard error ranging from ±5.7 to ±50.4 for paddy fields.

No significant effect of U (Site 1), AS (Site 1 and 2) or PS (Site 2) application on the cumulative ecosystem respiration was found (Table 8). When applying PS (Site 2), the

CO_2-C emissions en mass were the same from both treatments, that is to say they were not increase with the PS dose.

When applying CM (Site 1), the cumulative CO_2-C emissions were twice (CM-90-12) or five times (CM-170-12) those of the control. This is in accordance with the results of Hossain and Puteh (2013), who found that the application of chicken manure increased the cumulative CO_2 emissions by 121% compared to the control, as already stated for manures by Moore and Dalva (1993).

4.6. Global warming potential (GWP) and greenhouse gas intensity (GHGI)

4.6.1. Global warming potential and greenhouse gas intensity during the rice crop

The GWPs of the present study (Table 6) are in the same range as those found by Ma et al. (2013) and Wang et al. (2013). One key aspect here is that PS (Site 2) did not increase the GWP with respect to the control as AS (Site 2) did, while maintaining high yields (Table 6).

Negative GWP values suggest that the C sequestration exceeded the CO_2-eq emissions (Table 6).

Overall, the GWPs were high for the treatments with CM (Site 1), since CM application increased CH_4 and N_2O emissions (Table 6). Ma et al. (2013) and Wang et al. (2013) obtained coinciding results.

All fertilisation treatments resulted in high and similar yields (7 to 10 Mg ha^{-1}). The exception was for the AS-top-11 and the CM-170-12 treatments (both in Site 1; Table 6). In AS-top-11 (Site 1), there was a lack of available N once the losses were accounted for. In the CM-170-12 (Site 1) treatment, plants were damaged by a very serious attack of the fungus *Pyricularia oryzae*, revealing excessive N fertilisation (Yanni and Sehly, 1991).

Consequently, the GHGI relating GWP to grain yield was significantly ($p<0.05$) higher for the CM (Site 1) treatments than for the mineral fertiliser and control treatments (Table 6). The GHGI ranged from -1.57 to 5.18 kg CO_2-eq kg^{-1} grain yield (Table 5). A negative GHGI (consequence of the negative GWP) indicates equilibrium among yield, carbon sequestration into the soil and GHG emissions (Mosier et al., 2006, IPCC, 2013).

With the exception of the CM-90-12 and CM-170-12 treatments (Table 6), the rest of the GHGI of the present study are lower than those reported by Qin et al. (2010) and Shang et al. (2011) under continuous flooding conditions (2.06 to 3.22 kg CO_2-eq kg^{-1} grain yield).

The average GWP during the rice SP was as high as in the study by Linquist et al. (2015) with urea during the rice crop. However, at Site 1 the U application notably decreased the GWP since N_2O and CH_4 emissions were low (Table 8). The GWP during the rice SP was high for the treatments with CM as they significantly ($p<0.05$) increased CH_4 and N_2O emissions with respect to control and U-12 treatments (Table 8). The negative effect of CM on GHG emissions should be considered. At Site 2, PS applied at agronomic doses (~170 kg N ha^{-1}) kept the GWP to a minimum.

5. Conclusions

Under the tested Mediterranean conditions, the background application of U and AS, with an AS topdress led to the maximum rice yields (8-10 Mg ha^{-1}). Background fertilisation with organic fertilisers (CM at Site 1 or PS at Site 2) at a dose of 90 kg NH_4^+-N ha^{-1} led to similar yields.

During the rice crop, mineral N (AS and U) did not increase the cumulative N_2O emissions when compared to the control during the rice crop. Increasing the dose of CM (high C/N) tended to decrease the cumulative CH_4 emissions. Pig slurry (Site 2) application kept GHG emissions under control and ensured feasible yields.

During the rice crop, the maximum GWP was observed from the CM-90-12 dose (37 Mg CO_2-eq ha^{-1}; Site 1), although no significant differences between the CM and U treatments were observed. During the postharvest period, there were practically no N_2O emissions. The postharvest period was a net sink for CH_4, and CO_2 emissions only increased for the high CM dose (at Site 1).

The cumulative CH_4 emissions during the seedling period were about half to about 20% of the emissions during the rice crop. During the seedling period, AS (120 kg N ha^{-1}) and the application of PS (low C/N ratio) at an agronomic dose (91 kg NH_4^+-N ha^{-1}) at Site 2 did not increase CH_4 nor CO_2 emissions relative to the control. The CM (high C/N ratio; Site 1) applied at NH_4^+-N doses similar to those applied with PS (low C/N ratio; at Site 2), increased N_2O and CH_4 emissions with respect to the control.

During the SP, the GWP was the lowest for the AS and PS (low C/N ratio) treatments from Site 2, though not significantly different from the other treatments at the same site.

The application of PS at agronomic doses (~170 kg N ha^{-1}) could be considered a climate smart practice (FAO, 2013), as it allowed high yields (7.4 Mg ha^{-1}), the control of GWP (~6.5 Mg CO_2-eq ha^{-1}), and a 13% reduction in greenhouse gas intensity (GHGI) to 0.89 kg CO_2-eq kg^{-1} when compared to AS (1.02 kg CO_2-eq kg^{-1}).

The results of the present study suggest the need to reconsider if a 1% EF for N_2O emissions (as suggested by IPCC in the 2007 guidelines) is applicable to Mediterranean systems, or if different standards should be considered for specific areas and management practices.

Our findings show that organic manure (at agronomic dose) can be used in rice Mediterranean systems as fertilisers. As rice fields coexist with intensive animal rearing, our results support the nutrient recycling inside the agricultural systems. Furthermore it helps to maintain agricultural activity (due to the low cost of manures and slurries applications) in saline areas with environmental restrictions (Deltas and marshlands), and with waterlogged soils. These cultivated zones are very important in European environmental policies (EU Regulation 1765/92; NATURA 2000 network and the Ramsar Convention). These results reflect the specific conditions of a wide Mediterranean area that must be taken into account in global balances.

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